# Role of Korean red ginseng total saponins in rat infertility induced by polycystic ovaries

*Sok Cheon Pak, Ph.D.,*[a] *Sung Chul Lim, M.D., Ph.D.,*[b] *Seung-Yeol Nah, D.V.M., Ph.D.,*[c] *Jiae Lee, M.S.,*[d] *Joseph A. Hill, M.D., Ph.D.,*[e] *and Chun Sik Bae, D.V.M., Ph.D.*[f]

[a] New Zealand College of Oriental Medicine, Hamilton, New Zealand; [b] Department of Pathology and Research Center for Resistant Cells, Chosun University Medical School, Gwangju, South Korea; [c] Research Laboratory for the Study of Ginseng Signal Transduction and Department of Physiology, College of Veterinary Medicine, Konkuk University, Seoul, South Korea; [d] Creation and Love Women's Hospital, Gwangju, South Korea; [e] Fertility Centers of New England, Reading, Massachusetts; and [f] College of Veterinary Medicine, Biotechnology Research Institute, Chonnam National University, Gwangju, South Korea

**Objective:** To investigate the effect of Korean red ginseng total saponins (GTS) on ovarian morphology and nerve growth factor (NGF) expression in the ovaries, pituitary, and hippocampus.
**Design:** Polycystic ovary (PCO) rat model induced by estradiol valerate (EV).
**Setting:** University research laboratory.
**Patient(s):** Thirty sexually mature female Sprague-Dawley rats weighing 190–210 g.
**Intervention(s):** Female Sprague-Dawley rats (190–210 g) were separated into three groups: EV control (n = 10), EV plus GTS (n = 10), and oil control (n = 10).
**Main Outcome Measure(s):** Ovarian morphology and NGF protein expression.
**Result(s):** Polycystic ovary was fully developed in rats with a single intramuscular injection of EV. Increased expression of NGF was noted in the ovaries and the brain of rats with PCO. GTS administration attenuated NGF expression in the ovaries but not in the brain.
**Conclusion(s):** Our findings suggest a role for GTS in the regulation of NGF expression in female rats with PCO. (Fertil Steril® 2005;84(Suppl 2):1139–43. ©2005 by American Society for Reproductive Medicine.)

**Key Words:** Ginseng total saponins, polycystic ovary syndrome, nerve growth factor, estradiol valerate, immunohistochemistry

Polycystic ovary syndrome (PCOS) is one of the most common types of endocrinopathy among reproductive age women (1). This syndrome has been defined in a number of different ways since its initial description in 1935 (2, 3). The presence of a polycystic ovarian morphology has been recently included in the list of criteria for defining PCOS (4).

The major clinical features of PCOS are hyperandrogenism, anovulation and metabolic disturbances. Women with PCOS tend to have a higher luteinizing hormone/follicle-stimulating hormone (LH/FSH) ratio than normal women, which causes a disruption in the ovarian follicular development and anovulation (5). Furthermore, the disrupted estrogen levels along with the severe oligomenorrhea and amenorrhea can also result in endometrial hyperplasia and cervical cancer (6). The creation of an all-purpose animal model is complicated by the multifactorial etiology of PCOS (7–10). A rat model for PCOS based on the establishment of polycystic ovaries after an injection of estradiol ($E_2$) valerate (EV) has been described (7).

Received November 16, 2004; revised and accepted April 9, 2005.
This study was financially supported by research fund of Chonnam National University in 2003.
The first two authors contributed equally to this work.
Reprint requests: Chun Sik Bae, D.V.M., Ph.D., College of Veterinary Medicine, Biotechnology Research Institute, Chonnam National University, Yongbongdong, Bukgu, Gwangju 500-757, South Korea (FAX: +82-62-530-2809; E-mail: csbae210@chonnam.ac.kr).

The neurotrophin family including the nerve growth factor (NGF) reportedly involved the NGF receptor (11) and NGF mRNA (12) in ovulation and in the pathophysiology of PCOS. A polycystic ovary is reported to exhibit a high density of intraovarian nerve fibers that are associated with sympathetic hyperresponsiveness (12). In the rat ovary, NGF is mainly synthesized in the follicular wall cells (13). The activation of NGF might be involved in enhancing the norepinephrine outflow in an $E_2$-induced polycystic ovary (11).

Ginseng (the roots of *Panax ginseng* C.A. Meyer, Araliaceae) is a traditional Eastern Asian herbal medicine taken orally for a tonic and slowing down of the aging process. Ginseng is a popular dietary supplement throughout the world. The major active ingredients of ginseng are the ginseng saponins, which are composed of various ginsenosides (14, 15). To date, more than 30 ginsenosides have been identified (16). This study produced a murine PCO model using $E_2$ valerate to investigate the effect of Korean red ginseng total saponins (GTS) on the ovarian morphology and NGF protein expression in a rat PCO model.

## MATERIALS AND METHODS

### Animals

Sprague-Dawley female rats (n = 30) weighing 190–210 g and with regular 4-day estrous cycles were purchased from the Daehan Bio Link Co. (Taejon, South Korea). The rats

were housed under controlled conditions of temperature (25°C ± 1°C), with access to food and water ad libitum, and were cared for in accordance with the Animal Care Board at the Chonnam National University. Institutional Review Board approval was obtained before in vivo experiments.

### PCO Induction

Twenty rats received a single 4-mg IM injection of $E_2$ valerate (Sigma, St. Louis, MO) in 0.2 mL of sesame oil (Sigma) to induce a polycystic ovary, and ten received oil only for the control, as previously described (7). To obtain a fully developed polycystic ovary, a duration of 60 days after the injection was chosen.

### Ginseng Total Saponin

The GTS contained at least 11 glycosides, namely, Rb1 (18.26%), Rb2 (9.07%), Rc (9.65%), Rd (8.24%), Re (9.28%), Rf (3.48%), Rg1 (6.42%), Rg2 (3.62%), Rg3 (4.7%), Ro (3.82%), and Ra (2.91%), as well as other minor ginsenosides (20.55%). The GTS was a kind gift from the Korea Ginseng and Tobacco Research Institute, Taejon, Korea. The GTS was dissolved in saline and administered intraperitoneally.

### Study Protocol

The animals were randomized into the two treatment groups in a blind manner: ten rats in the EV control group and ten in the GTS-treated group (and ten in the oil control group). The GTS-treated EV group rats were administered 100 mg/kg GTS intraperitoneally every other day for 60 days, beginning 1 day after the EV injection.

### Ovarian Morphology

All 30 rats were killed by transcardial perfusion with chloral hydrate (500 mg/kg) anesthesia containing a 4% paraformaldehyde solution in 0.1 mol/L of sodium cacodylate buffer and 4% sucrose added at pH 7.4. The dissected ovaries were bisected midsagittally and placed in the same fixative overnight at room temperature. The ovaries were then totally embedded in paraffin. The samples were sectioned at 4 $\mu$m and stained with hematoxylin and eosin. The number of follicles containing an oocyte with a nucleus were counted and analyzed by the same individual blinded to the treatment group. If ovum degeneration or at least one pyknotic granulosa cell was observed, the follicle populations were classified as being atretic. The morphologic characteristics of follicular atresia included scattered pyknotic nuclei in the granulosa cell layer, detachment of the granulosa cell layer from the basement membrane, fragmentation of the basal lamina, and the presence of cell debris in the antrum of the follicle.

### Nerve Growth Factor Measurement by Immunohistochemistry

The dissected ovary, pituitary, and hippocampus were stored in fixative at room temperature for 2 days. The fixed tissue samples were embedded in paraffin for microtome slicing into 4-$\mu$m thick slices, and the sliced tissue sections were mounted on X-tra slides (Surgipath, Richmond, VA). To observe the immunohistochemical reaction to NGF, the slides were deparaffinated and hydrated by immersing sequentially in xylene and decreasing concentrations of ethanol (100%, 95%, 90%, and 80%) for 5 minutes each followed by distilled water for 10 minutes. The level of antigen retrieval was enhanced by pretreating the sections in a microwave (Pelco laboratory microwave oven; Ted Pella, Redding, CA) in 0.01 mol/L citrate buffer (pH 6.0) 3 times for 5 minutes at 360 W. The endogenous peroxidase activity was blocked with 3% hydrogen peroxide in methanol for 15 minutes. After each step, the sections were washed with 50 mmol/L Tris buffered saline (TBS, pH 7.5) 3 times for 10 minutes each. The tissue sections were then covered with a blocking antibody for 10 minutes and incubated overnight at 4°C with a rabbit antimouse NGF antibody (clone 2.5s; Serotec, Kidlington, Oxford, UK) at a dilution of 1:50. For each case, a corresponding section was incubated in TBS without the primary antibody as a control for nonspecific staining. The biotinylated rabbit antimouse secondary antibody was added for 10 minutes, which was followed by adding the avidin-biotinylated peroxidase complex for an additional 10 minutes. After washing, the sections were stained with 3-amino-9-ethyl carbazole (AEC; Vector Laboratories, Burlingame, CA), counterstained with Mayor's hematoxylin and mounted. The positive control for NGF was the cerebral cortical neurons of the rat. Instead of the primary antibody, TBS was used as the negative control.

### Analysis and Interpretation of Immunohistochemical Staining

Two independent observers without any knowledge of the experiments assessed the stained sections. Consensus scores were assigned for each case by reviewing the slides with scoring discrepancies. The NGF was decoded according to the staining intensity and the number of positive stained cells as follows: 0, negative staining; 1, a few positive staining; 2, diffuse weakly positive staining; 3, moderately positive staining; 4, strongly positive staining.

Each score was added together and mean ± SD was calculated in each group.

### Statistics

All the values for the weights of body and ovaries, and the scores for the NGF immunostaining in the ovary, pituitary, and hippocampus were compared using analysis of variance. The significance level was .05.